A Study of Acetyl-CoA Decarbonylase/Synthase Using X-Ray Magnetic Circular Dichroism at Beam Line 4.02 of the ALS

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INTRODUCTION

X-ray magnetic circular dichroism (XMCD) spectroscopy provides a unique opportunity to study spin and oxidation states of dilute transition metals in metallo proteins. Advantages of the technique include element selectivity and high sensitivity.
The XMCD signal is given by the difference in absorption between right and left circular polarized X-rays [1]. XMCD probes the population of the magnetically split levels by measuring the difference in absorption between right and left circular polarized X-rays. Since in paramagnetic systems this population is given by Boltzmann statistics, XMCD requires high fields and low temperatures.
Applying the sum rules to the measured spectra allows determining the spin and the orbital angular momentum of the metal centers [2]. Furthermore, one can observe the signal as function of the magnetic field to get magnetization curves of the system. This allows to extract the total angular momentum and the g-factor of the specific metal sites by fitting a Brillouin function. This also provides information, if various chemical species of the same element are present.
We have studied the active site of the enzyme acetyl-CoA decarbonylase/synthase (ACDS) using this method. ACDS contains 5 protein subunits and catalyzes the cleavage (or synthesis) of acetyl-CoA [5]. In the current model, the active site A cluster (which is located on the ACDS β subunit) contains an Fe-S cluster with a Ni atom bridged to it [3]. During the catalytic cycle of ACDS the Ni and the Fe sites change the oxidation state. An important step to understand the catalyzes is to understand the various steps in the catalytic cycle.

EXPERIMENTS

With our current setup located at the elliptically polarized undulator beam line of the ALS we can study systems with a metal concentration of 500 ppm and below utilizing a commercial 30 element Ge detector. Our endstation hosts a 6 Tesla superconducting magnet cooled with liquid helium. The sample is located in the bore of the magnet and is attached to a separate pumped ^{4}He cryostat providing sample temperatures of 2.2 K [4].
The proteins are prepared in a dried film on a sapphire disk in a glove box. The sample is transferred to the experimental chamber and cooled to base temperature in a specially designed capped sample holder.
We prepared ACDS β sununit in 2 states important for the catalyzes. One state was obtained by reducing the protein with Ti citrate and another was obtained treating the reduced protein with CO.
The magnetic response was studied at the Ni and the Fe sites recording the respective L-edges with circularly polarized light at 2.2K and 6T.

RESULTS AND DISCUSSION

Ti citrate reduced ACDSβ shows a strong magnetic signal in the Ni as well as in the Fe spectrum.

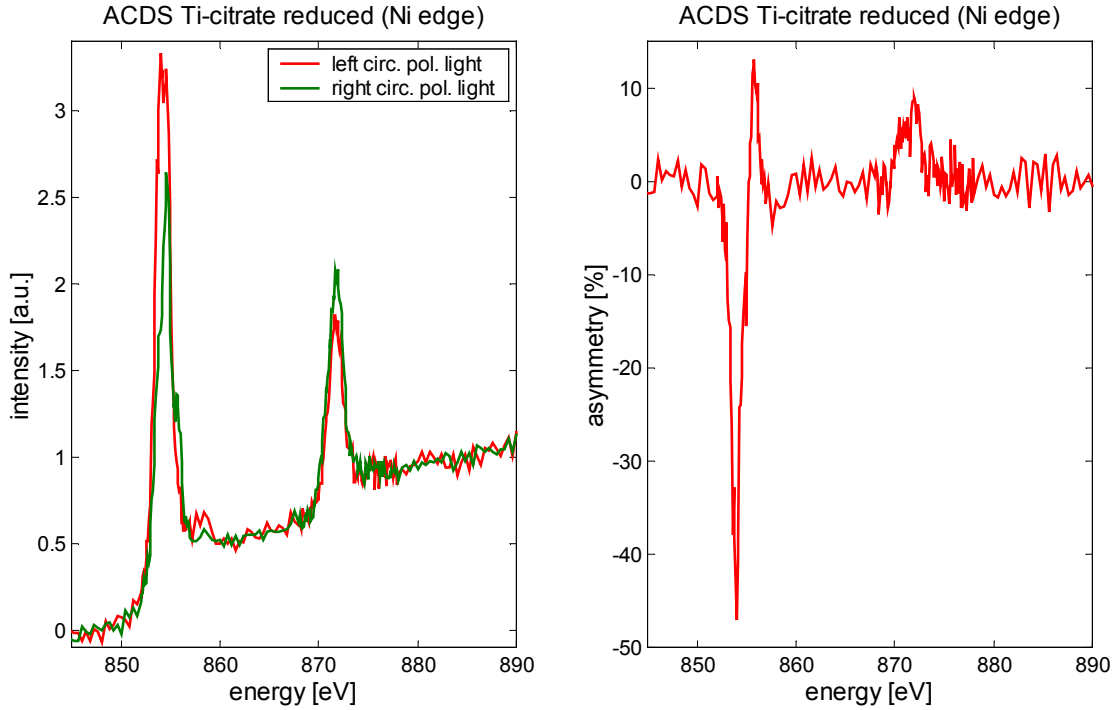

Figure 1a and 1b: L-edge spectra with right and left circularly polarized light (a). Difference of with right and left circularly polarized light spectra and normalized to maximum of L-edge spectrum (b).

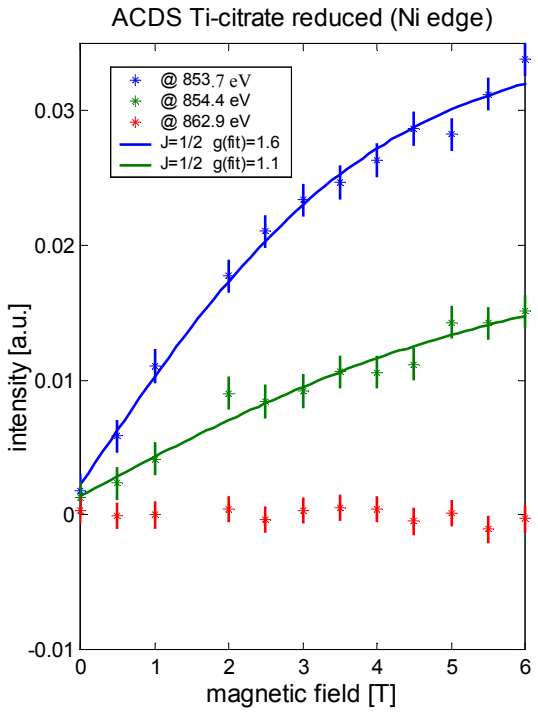

Figure 2: Magnetization curves at different excitation energies. The red curve was taken between the L3 and L2 line and shows no signal

Figure 1a shows the L-edge spectra of Ni for left and right circularly polarized light. Figure 1b shows the XMCD signal taking the difference spectrum between left and right circularly polarized light normalized with the maximum of the L-edge spectra leading to an XMCD effect of around 50%. Magnetization curves were taken at various excitation energies as shown in figure 2. One curve was measured at 862.9 eV just between the L3 and L2 lines. Since no magnetic response is expected at this position this curve determines the base line. Another set of curves were taken at two positions of the L3 line. Fits of the Brillouin function to the data results in different g-values. This indicates the presence of different chemical species of Ni in the sample with different magnetic properties. The measured spectrum is a superposition of the spectra of the different species. A similar behavior was observed for the magnetization curves taken at the Fe edge. The L3 edge is

showing a double peak feature with both peaks exhibiting a strong magnetic response. The maximum XMCD signal is about 35%.
Treating the sample with CO changes the resulting spectra dramatically. The magnetic signal on the Ni site disappears almost completely. A residual XMCD signal of 10% is observed. Also the XMCD signal of the Fe is drastically reduced to about 20%. The double peak feature at the L3 line also disappears. A magnetization curve was taken at the excitation energy of the maximum Fe-XMCD signal revealing a magnetization close to saturation. This rules out that the XMCD signal disappeared due to insufficient thermal contact.

ACKNOWLEDGEMENTS

The NIH through Grant No. GM4430 and the Department of Energy, Office of Biological and Environmental Research supported this work. For excellent support we like to thank Tony Young, Elke Arenholz, and Rene Delano and the staff of the ALS.

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